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CO-releasing Metal Carbonyl Compounds as Antimicrobial Agents in the Post-antibiotic Era

The possibility of a “post-antibiotic era” in the 21st century, in which common infections may kill, has prompted research into radically new antimicrobials. CO-releasing molecules (CORMs), mostly metal carbonyl compounds, originally developed for therapeutic CO delivery in animals, are potent antimicrobial agents. Certain CORMs inhibit growth and respiration, reduce viability, and release CO to intracellular hemes, as predicted, but their actions are more complex, as revealed by transcriptomic datasets and modeling. Progress is hindered by difficulties in detecting CO release intracellularly, limited understanding of the biological chemistry of CO reactions with non-heme targets, and the cytotoxicity of some CORMs to mammalian cells.

It is axiomatic that metal ions are essential in biology, but also toxic in unregulated concentrations or locations. A corollary is that selectively toxic metal compounds (such as compounds of silver for infections resulting from burns and bismuth in fighting Helicobacter pylori) have long been used as antimicrobial compounds, antiseptics, and disinfectants (1). It is therefore paradoxical that metal compounds are the most abundant class of compounds for delivering carbon monoxide (CO) for therapeutic purposes in higher organisms. Although CO is a respiratory poison, it has “come of age” since the discovery that CO is a cytoprotective and homeostatic molecule and a vasodilator, anti-inflammatory, anti-apoptotic, and anti-proliferative agent (2–4). The biological chemistry of CO is relatively simple (when compared with O2 and the “gasotransmitters” NO and H2S) (5, 6). Its most important property is reaction with metals, famously ferrous heme proteins, although some heme-independent reactions are known, such as binding to iron in hydrogenases (7) and to binuclear copper sites, for example in hemo-cyanins (8). In CO dehydrogenase, which oxidizes CO to CO2, CO interacts with the nickel ion in one of the metalloclusters (“C-cluster”) (9). Here we review the effects of CO and CO-releasing molecules (CORMs) on microorganisms, experiments that demonstrate the potential of CORMs, and highlight problems and prospects.

Development and Applications of CORMs

Resistance to antibiotics now threatens the effective prevention and treatment of microbial infections (10). This scenario is not an apocalyptic fantasy, and has promoted research into the development of new antimicrobial agents. CORMs, originally developed for therapeutic delivery (3, 4), have recently been investigated for their antimicrobial activities, initially presumed to be mediated by CO. If the delivery of CO to targets could be controlled and enhanced, it might be toxic to microorganisms; indeed, CO-supplemented gas atmospheres preserve meat from bacterial spoilage (11). However, microbes may also be relatively insensitive to the gas. Airborne bacteria survive high urban CO concentrations (12), and bacterial cultures may be bubbled with the gas (13); 250 ppm of CO is not toxic (14). Furthermore, CO per se is not selectively toxic to microbes; it is tolerated at about 3 mg/kg for 1 h in humans, and no toxic effects are evident in animal models at efficacious doses of the gas (when carbonmonoxymethemoglobin levels reach ~20%) (4).

The key to the use of CORMs as antimicrobials is that they are far more toxic to microbes than is CO, but the basis of this toxicity is poorly understood. Mann (3) authoritatively reviews the discovery and development of CORMs. Early biological studies investigated binding to heme proteins, vasodilation, inhibition of NO production by macrophages (because CO deactivates inducible NO synthase while activating guanylyl cyclase), and survival of animals after organ transplantation (3, 15). Antimicrobial effects were not considered. Numerous CORMs have been reported and synthesized, but here and in Table 1, we describe only those that have been used against microbes or hold particular promise (16–26). Two ruthenium compounds have been extensively used: CORM-2 and CORM-3. The former has long been commercially available, but the latter has only recently been marketed. Although CORM-2 is soluble in dimethyl sulfoxide, the outstanding merit of CORM-3 is water solubility (27, 28). However, it has complex solution chemistry, and many aspects of its biological fate and CO release remain unresolved. In water, CO release is slow so that solutions can be prepared and administered with ease, but CORM-3 releases CO rapidly in the standard assay that uses ferrous myoglobin as acceptor, leading to the description of CORM-3 as a rapid CO releaser (27) (but see below).

Other CORMs are covered below where they have been used as antimicrobial agents. Newer compounds with desirable therapeutic effects are constantly appearing, but few have been

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** This article contains supplemental Fig. 1 and supplemental Table 1.

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The abbreviations used are: CORM, CO-releasing molecule; iCORM, inactive form of CORM from which CO release cannot be detected; HO-1, heme oxygenase 1; NAC, N-acetylcysteine; ROS, reactive oxygen species; iNOS, inducible NO; photoCORM, photoactivatable CORM; tryptoCORM, trypto-phan-derived manganese-containing complex; IM, inner membrane; OM, outer membrane; TF, transcription factors.

2 The abbreviations used are: CORM, CO-releasing molecule; iCORM, inactive form of CORM from which CO release cannot be detected; HO-1, heme oxygenase 1; NAC, N-acetylcysteine; ROS, reactive oxygen species; iNOS, inducible NO; photoCORM, photoactivatable CORM; tryptoCORM, trypto-phan-derived manganese-containing complex; IM, inner membrane; OM, outer membrane; TF, transcription factors.
tested microbiologically (29, 30). Of particular interest are CORMs in which the CO release can be precisely controlled both spatially and temporally, either by triggering the inactive “prodrug” with light (photoCORMs) (31) or by enzyme activation (32).

### Analytical Methods as a Bottleneck in Understanding CORM Toxicity

CO is generally assayed in environmental, clinical, or experimental situations by measuring the characteristic absorbance spectrum on reaction with myoglobin (above), or by GC-thermal conductivity detection of CO (41). Refinements to the myoglobin assay were proposed (42), but we demonstrated that it is the reducing agent for myoglobin, sodium dithionite, that promotes CO release (43); CO is not released from CORM-3 in the absence of the reductant (43). It might be explained by the fact that dithionite is not pure and contains a significant quantity of sulfite, which is in equilibrium with sulfur dioxide, a good ligand for transition metals. This fits with the observation that, on dissolution in buffers in a closed vial, only CO$_2$, resulting from the water-gas shift reaction, can be detected (by GC) (33). The mechanism of CO release from CORM-3 remains unknown as its chemistry is complex (28), but decomposition products of CORM-3 react with exposed His residues on protein to give metallocarbons that spontaneously release CO (44). Thus, in biological situations where dithionite (or sulfite, metabisulfite, or perhaps other species) are absent, the myoglobin assay overestimates the rate of CO release. Likewise, CORM-3 does not release CO to the purified flavohemoglobin (Hmp) when reduced with NADH but does so in the presence of dithionite (45). These findings probably explain the discrepancy noted between the myoglobin assay and the CO electrode (22), previously attributed to the need for certain CORMs to interact “with biological components to trigger the release of CO” (22). An alternative assay that obviates the need for dithionite uses oxyhemoglobin (43). Such globin assays could in principle be applied to CO assays within bacteria; indeed Escherichia coli Hmp expressed at high copy number is a sensitive monitor of CO liberated inside bacteria from CORMs (45).

Newer methods with unrealized potential include FTIR and photothermally induced resonance to detect an organometallic carbonyl compound (not a CORM) in breast cancer cells (46). More promising is Raman microspectroscopy to detect a manganese CORM [Mn(tpm)(CO)$_3$]Cl (tpm = tris(1-pyrazolyl)methane) in colon cancer cells (47). A genetically constructed fluorescent probe (COSer) comprises the CO binding selectivity of CooA, a dimeric CO-sensing heme protein from Rhodospirillum rubrum, and a fluorescent peptide to report conformational changes on binding CO (48). Transfection of HeLa cells with COSer allowed intracellular imaging of CO after treatment with CO or 1–10 $\mu$M CORM-2. A new fluorescent probe (COP-1) based on palladium-mediated carbonylation allowed selective CO detection in cells after CORM-3 treatment (49). COP-1 has also been used in vitro to demonstrate CO release from a photoCORM in the presence of endothelial cells (35). Zobi et al. (50) have shown via synchrotron FTIR spectromicroscopy that a photoactivated CORM conjugated to vitamin B$_{12}$ is taken up by fibroblasts. A photoCORM that is also luminescent could be tracked by confocal fluorescence microscopy (51). These methods have not been tested in bacteria, but the attainable spatial resolution appears at present inadequate for subcellular localization.

### Table 1: CORMs referred to in this review

<table>
<thead>
<tr>
<th>CORM</th>
<th>Chemical name/Reference</th>
<th>Structure</th>
<th>CO release characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALF021</td>
<td>Bromo(pentacarbonyl) manganese (16)</td>
<td>Released spontaneously in solution</td>
<td></td>
</tr>
<tr>
<td>ALF062</td>
<td>Tetraethylammonium molybdenum pentacarbonyl bromide (16)</td>
<td>Released spontaneously in solution</td>
<td></td>
</tr>
<tr>
<td>ALF070</td>
<td>Tricarbonyl<a href="II">di(8-thiogalactopyranosyl)boron(V)</a> (17)</td>
<td>1 mol of CO/mol CORM</td>
<td></td>
</tr>
<tr>
<td>CORM-2</td>
<td>Tricarbonyl<a href="II">di(8-thiogalactopyranosyl)boron(V)</a> dimer (16), (18)</td>
<td>Ligand substitution; Promoted by dithionite; 0.7 mol CO/mol CORM</td>
<td></td>
</tr>
<tr>
<td>ALF035</td>
<td>Ru(CO)$_3$Glucose (19), (20), (21)</td>
<td>Promoted by dithionite; 1 mol CO/mol CORM</td>
<td></td>
</tr>
<tr>
<td>CORM-371</td>
<td>[Mn$_2$N$_2$][Mn(CO)$_3$(thiacetate)$_2$] (22)</td>
<td>1 mol CO/mol CORM; Half time = 20.2 min</td>
<td></td>
</tr>
<tr>
<td>CORM-A1</td>
<td>Sodium boroncarbide (23)</td>
<td>Spontaneous release of CO; pH- and temperature-dependent; Half time = 21 min</td>
<td></td>
</tr>
<tr>
<td>CORM-401</td>
<td>[Mn(CO)$_3$(S$_2$C$_2$NMe(CH$_2$CO)$_2$H)] (24)</td>
<td>1 mol CO spontaneously; $\leq 3.2$ mol CO/mol CORM by ligand substitution</td>
<td></td>
</tr>
<tr>
<td>photoCORM - USC-CN029-31</td>
<td>Mn(CO)$_3$(tpza-e$^+$N)$^+$ (25)</td>
<td>Release of CO upon irradiation; 365 nm = 2.2 mol CO/mM CORM</td>
<td></td>
</tr>
<tr>
<td>cryptopCORM</td>
<td>Tricarbonyl acetominitrile (L-2-Amino-3-(1H-indol-3-yl)propanoic acid (26)</td>
<td>Release of CO upon irradiation; 400 nm = 2 mol CO/mol CORM; 465 nm = 1.4 mol CO/mol CORM</td>
<td></td>
</tr>
</tbody>
</table>
CO Metabolism in Microorganisms: Implications for Pathogenesis

To understand the possible mechanisms of action of CORMs, it is clearly important to appreciate how CO per se impacts on microorganisms. DNA replication is inhibited by CO (52), and the inhibition by CO of respiratory oxidases and globins at heme targets has been known since the days of Warburg and Keilin (reviewed in Ref. 53). However, CO also binds to the di-iron site in bacterial NO reductases (54, 55) and to iron, copper, and nickel sites in certain microbial proteins, notably CO dehydrogenase (see above).

The relationship between CO and disease is complex, but clues come from the observation that cigarette smoking and CO, a component of smoke, have anti-inflammatory effects against ulcerative colitis (56). However, the major CO source in mammals is CO endogenously produced by heme oxygenase (HO)-1 (57). Several bacteria also possess HO enzymes that function to degrade heme that is imported for use as an iron source (58, 59). HO activity contributes to pathogenesis in certain bacteria by scavenging iron from heme (58, 60).

There is extensive literature on sensing of gases (O2, NO, CO) by mycobacteria and its role in dormancy. Mycobacterium tuberculosis infection of macrophages and mice induces host HO-1 expression (61). The CO thus produced, together with iNOS-derived NO, stimulates expression (via the heme two-component sensor kinases DosS and DosT and the cognate response regulator DosR) of the bacterial dormancy regulon, a group of about 50 genes with diverse functions (61, 62). A recently described CO resistance gene (cor) in M. tuberculosis appears important in dictating the outcome of the host-bacterium battle; the virulence of a cor mutant is attenuated in a mouse model of tuberculosis. Expression of the Cor protein in E. coli is claimed to rescue it from CO toxicity, but the resistance demonstrated was to CORM-2 not CO (63).

The HO (Hmx1) of the pathogenic yeast Candida albicans and its product, CO, also contribute to pathogenesis (64); mutagenesis of the HMX1 gene results in decreased virulence in murine candidiasis, whereas exposure of mice to therapeutic levels of CO increases C. albicans virulence. Inhaled CO partially reverses the virulence defect of the null strain, and so the data are consistent with CO-mediated suppression of acute host inflammatory responses (64).

Heme Oxygenases of Mammalian Cells: Implications for Infection

Mice deficient in HO-1 are susceptible to oxidant-induced tissue injury, but administration of CO to animals exposed to endotoxin decreases inflammation. HO-1- or CORM-2-derived CO rescues mice from lethal endotoxemia and sepsis (65). However, the role of CO in tackling a pathogen is less clear (66, 67). Indeed, suppression of inflammation might compromise the immune system. Otterbein et al. (68) showed that CO gas enhances phagocytosis, and Chung et al. (69) showed that CO derived from HO-1 enhanced the host defense response to polymicrobial sepsis in mice and contributed to bacterial clearing by stimulating phagocytosis.

Enterohemorrhagic E. coli (EHEC) stimulate the rapid inducible expression of the human enterocyte HMOX-1 gene that encodes HO-1, and its activity is a critical modulator of the innate immune response (70). Because HO-1 activity inhibits iNOS induction, EHEC effectively suppresses NO generation, and thus host antimicrobial activity. The CO donor CORM-2 also inhibited iNOS mRNA expression, thus identifying CO, not bilirubin (another product of HO-1 activity), as the effective species (but see caveats below regarding the non-equivalence of CORMs and CO). Up-regulation of HO-1 was shown to offer protection in mice against infection by Mycobacterium avium or M. tuberculosis, whereas HO-deficient mice were more susceptible (71). Thus, HO-1 may be an important cytoprotective protein in sepsis and inflammation.

CO is also implicated in the pathogenesis of Clostridium difficile. Inhibition of host HO activity by administering Zn protoporphyrin IX to mice exacerbated the histopathological alterations elicited by C. difficile toxin A; conversely, pretreatment of mice with a CO donor (dimanganese decarboxyl) reduced the effect (60).

In a recent study, enteric microbiota isolated from pathogen-free mice induced production of HO-1 in colons of wild-type mice but not in colitis-prone interleukin (II)10−/− animals (72). However, pharmacological induction of HO-1 by Co(III) protoporphyrin IX chloride protects interleukin− mice from microbiota (Salmonella enterica serovar Typhimurium)-induced colitis. Moreover, HO-derived CO reduced the numbers of live bacteria recovered from various organs, whereas knockdown of HO-1 in macrophages impaired bacterial activity. Thus, HO-1 and CO ameliorate intestinal inflammation through promotion of bacterial clearance, in part explained by promoting bactericidal activities of macrophages (72, 73).

Recently, Wegiel et al. (14) have proposed that ATP, acting as a pathogen-associated molecular pattern, which is recognized by innate immune cells, is released from viable bacteria in the presence of CO and triggers activation of the macrophage, inflammasome, and IL-1β secretion. Curiously, it is suggested that an oxidase binds CO “to compel ATP generation much like that observed in the ATP synthase mutant” (14). However, Gram-negative bacteria are not known to possess periplasmic ATP or to have mechanisms for secretion, so the observed effect is poorly understood.

The Antimicrobial Effects of CO and CORMs in Vitro and in Vivo

In many respects, CO is an attractive candidate for an antimicrobial molecule; it is rarely metabolized and “stable,” is adequately water-soluble, traverses cell membranes (5), and is a molecule that is naturally generated in mammals, plants, and certain microorganisms by HO (supplemental Table 1). There is a rapidly growing literature on the diverse antimicrobial effects of CORMs on bacteria (Fig. 1). Nobre et al. (16) first described the use of CORMs as antimicrobial agents. CORM-2 and CORM-3 and compounds from Alfa, Inc. (ALF021, bromo(pentacarbonyl)manganese, and ALF062, tetraethylammonium molybdenum pentacarbonyl bromide) (Table 1) were tested against laboratory strains of E. coli and Staphylococcus aureus (16). For example, killing of greater than 20% was
achieved within 1 h with 250 μM CORM-2, and more variable killing was achieved with 400 μM CORM-3. Control experiments with hemoglobin to sequester CO and the use of inactive forms of the CORMs or solvent-only controls suggested that CO release was the major cause of killing, yet a flux of CO gas was markedly less effective than the CORMs. Interestingly, CO was not detected in media to which the CORMs were added, implying that CO release occurs only intracellularly or that the CO liberated extracellularly escapes from the culture.

Three important studies indicate the potential for CORM-elicited antimicrobial effects in animal models. Chung et al. (69) showed that CO from HO-1 enhanced the response to sepsis in mice and stimulated phagocytosis, an effect mimicked by injection of CORM-2. Second, CORM-2 and CORM-3 were effective in protecting immunocompetent and immunocompromised mice when injected following Pseudomonas aeruginosa-induced bacteremia (20), but CORM-371 was not (22). The data suggest a direct bactericidal action rather than stimulation of phagocytosis. Third, activity of ALF492 (tricarbonyldichloro(thiogalactopyranoside)Ru(II)) (Table 1) was demonstrated (17) in mice against the protozoan parasite Plasmodium falciparum; the injected compound protected mice against experimental cerebral malaria and acute lung injury without formation of carbonmonoxyhemoglobin. The protective effect was CO-dependent, and the CORM elicited expression of HO-1, thus amplifying the protection. ALF492 was also shown to be an adjuvant to the established antimalarial compound artesunate (17).

However, most recent studies have used in vitro methods and cast doubt on our understanding of the fundamental modes of action, especially the suggestion that CORMs exert antimicrobial activities solely through CO release. Several authors have reported that CORMs are more effective antimicrobial agents than is CO (16, 21). For example, 100 μM CORM-3 was effective against P. aeruginosa in vitro (20), but CO gas (860 μM) was not. Importantly, even 10 μM CORM-3 was effective against antibiotic-resistant clinical isolates but was not inhibitory to macrophage survival.

Recently, CORMs that release CO only on illumination have been developed and tested as antimicrobial agents. The first such study describes a manganese CORM (Table 1) that acts as
a stable prodrug in the dark, whereas 365 nm illumination leads to CO release to myoglobin (25). Only after irradiation is the compound toxic to E. coli, in which CO-ligated terminal oxidases can be detected following internalization of the compound. This compound has the advantage of a well-defined inactivated form of CORM (iCORM) (25). Similarly, a tryptophan-derived manganese-containing complex (tryptoCORM) that releases 1.4 mol of CO on irradiation at 465 nm, and 2 mol at 400 nm, is toxic to E. coli but not to macrophages (26).

Concerns over the inexorable spread of antibiotic resistance and the paucity of new antimicrobial drugs have led to studies not only of CORMs as antimicrobials in their own right against antibiotic-resistant clinical isolates (20, 74), but also as adjuvants to established antibiotics, a common practice in clinical therapy (i.e. combination therapy) (supplemental Fig. 1). In one study, sub-lethal doses of CORM-2 were combined with metronidazole, amoxicillin, and clarithromycin and found to potentiate antibiotic effects on clinical isolates of H. pylori (75). Two mechanisms of action were reported: inhibition of respiration and of urease activity. CORM-2 decreased the measured minimal inhibitory and minimal bactericidal concentrations for all antibiotics. Similarly, CORM-2 acts as an adjuvant to tobramycin against P. aeruginosa biofilms (76). In neither of these studies was it reported whether the effects of CORM-2 and antibiotics together were truly synergistic or merely additive, as assessed by standard fractional inhibitory concentrations (77). However, these potentiating effects observed with CORMs have not been reported to our knowledge with CO gas, although NO and H2S have been shown to confer some defense against antibiotics (78).

Transcriptomic and Global Impacts of CORMs

Transcriptomic approaches have been highly informative and emphasized the complexity of the CORM response. In the first study (21), batch cultures of E. coli were used to explore exposure to sub-inhibitory (30-100 μM) concentrations of CORM-3, aerobically and anaerobically. The down-regulation of operons encoding key respiratory complexes (cytochrome bo' and several dehydrogenases) was striking. Interestingly, the cydAB genes encoding cytochrome bd-1, an inhibitor-resistant terminal oxidase with a high oxygen affinity, were slightly up-regulated. The genes most highly up-regulated were involved in metal homeostasis, especially spy, which encoded a periplasmic stress-response chaperone. Probabilistic modeling of the comprehensive datasets (21) identified global transcription factors that are potential CO targets or sensors, notably the respiratory metabolism regulators ArcA and Fnr. However, a similar study using 250 μM CORM-2 (partly bactericidal within 30 min (16)) revealed (79) a gene set with few similarities to the CORM-3 study, but up-regulation of spy and down-regulation of some respiratory operons were observed.

A more rigorous and reproducible approach to transcriptomics is provided by chemostat (continuous) culture in which all growth conditions, including growth rate, are maintained over long periods, thus avoiding growth rate-dependent changes in gene expression (80). Mclean et al. (81) used not only CORM-3 but also the inactivated iCORM-3 (from which negligible CO release can be shown) to dissect the effects of CO release and other consequences of the E. coli response in a chemostat. Transcriptomics revealed that the response to iCORM-3 is lower than to CORM-3, but that numerous processes are affected by both compounds, including energy metabolism, membrane transport, motility, and the metabolism of sulfur-containing species, including cysteine and methionine.

There is controversy regarding the roles of reactive oxygen species (ROS) and antioxidants in the antibacterial effectiveness of CORMs; the evidence in favor is given in Ref. 59. It is established that inhibition of bacterial oxidase activity by CO can lead to higher ROS levels (82), for example from exposed flavins in NADH dehydrogenase (83). However, Tavares et al. (84) propose the direct involvement of ROS in the toxicity of CORM-2 and ALF062 to E. coli; both promote the production of reactive oxygen species, an effect blocked by antioxidants. Mutations in superoxide dismutase or catalase exacerbated CORM toxicity, and CORM-2 induced expression of the DNA repair/SOS system recA and raised levels of free iron in cells. In contrast, treatment of P. aeruginosa with three CORMs did not change ROS production (22).

Certain antioxidants (N-acetylcysteine (NAC) and ascorbic acid) suppress H2O2 levels, and NAC, cysteine, and reduced (but not oxidized) glutathione reverse CORM-3-mediated inhibition of bacterial growth and respiration (20, 81). Glutathione and cysteine also prevented killing of H. pylori by CORM-2, but ROS could not be detected and ascorbic acid did not prevent the antimicrobial effect of CORM-2 (75). Thus, the basis of the effects of these sulphhydryl compounds remains poorly understood but is important because many are intracellular compounds and might promote or modulate CO release in vivo (81). Significantly, the effects of antioxidants on CORM toxicity may be linked, not only to counteracting the intracellular toxic effects, but also to the uptake of the CORM. Jesse et al. (85) found that NAC, widely used to abrogate CORM effects, not only protected respiration from CORM-2 or CORM-3 but also dramatically reduced (5–8-fold) CORM uptake.

The transcriptomic evidence is contradictory. Many genes implicated with intracellular redox stress were reported in E. coli by some (79) but not all (21) authors. The genes spy, spb, metF, and htpX, seen by us (21) are described in Ref. 59 as “associated with the generation of intracellular oxidative stress.” However, the up-regulation of spy (the most dramatically changed gene: 26–100-fold (21), not 3-fold as reported in Ref. 59) is attributed not exclusively to oxidative stress but to hypochlorite-induced membrane disruption (86).

How Significant Is Respiratory Blockade in Determining CORM Effectiveness?

Cellular respiration is inhibited by CO gas in vitro and in cells via endogenous HO activity (87, 88). Although reaction of CORM-derived CO with intracellular ferrous hemes has been reported consistently (e.g. Refs. 21, 45, and 82), and functionally distinct oxides have differential sensitivities to CORMs (85), inhibition of respiration is not the only factor affecting the bacterial activity of CORMs (22). CORMs may be toxic under anoxic conditions in the absence of respiration (16, 20, 21). Indeed, in mitochondria, CORMs may inhibit respiration (87,
89) or not (90–92). The reported uncoupling of mitochondrial respiration by CORM-3 (deduced from stimulated oxygen consumption rates) (90–92) and by CORM-401 in cardiomyocytes (93) is relevant to bacteria because CORM-3 at low doses also stimulates respiration in E. coli (94). However, classical uncoupling appears not to be the cause because proton translocation quotients and proton backflow rates are unaffected by CORM-3 (94). The stimulatory effects may arise from reaction of CO or CORMs with membrane channels as described in mammalian cells (95–97).

What Is the Mechanism of CORM Activity against Microorganisms?

Wherever an answer to this key question has been sought, investigators have found that bacteria accumulate CORMs (16, 21, 81, 85), that CO is bound to identifiable targets (i.e. heme proteins), and CO causes global changes in gene expression and cell function (Fig. 1). Furthermore, CO gas (as evidenced from data with HO-derived CO in vivo; see above) also perturbs microbial behavior. However, although CORMs were originally developed for safe and reproducible delivery of CO in mammals, the evidence to hand, summarized above, makes it improbable that CO delivery alone is the sole basis of the antimicrobial effects of CORMs. What evidence supports this bold claim? (a) Saturating solutions of CO gas barely perturb bacterial growth. (b) Bacteria demonstrate multiple transcriptomic changes to CORM-3 that cannot be understood in terms of known CO biochemistry. (c) Bacteria respond to iCORM-3 from which no, or negligible, CO release can be demonstrated in vitro. (d) Critically, cells lacking all hemes are also inhibited by CORM-3 and reveal multiple transcriptomic changes (101). (e) Finally, other compounds of Ru are taken up and have antimicrobial properties, although they are not CORMs (e.g. Refs. 1 and 98). We have suggested (94) that a CORM functions as a “Trojan Horse,” in which the metal carbonyl is the “horse,” delivering a cargo of toxic CO; it is equally conceivable that the toxic cargo is the metal fragment and that CO potentiates uptake.

Future Prospects

Realizing the future potential for CORMs relies on greater understanding of the modes of action of current CORMs and the development of improved compounds with clinical compatibility, for example by making biocompatible CO carriers (99). In the post-antibiotic era, there appears to be potential for adjuvant/combination therapy in which CORMs can minimize usage of established antibiotics or reduce the concentrations needed to treat antibiotic-resistant “superbugs.” Apart from methodological advances in detecting CO, a “CO-quenching” agent would allow the essential dissection of the antibacterial roles of the CO per se and the CORM; a water-soluble complex has been tested as a CO “stripper” in a rat model (100). Other areas of focus should be improved iCORMs that can be reproducibly prepared and whose chemistry is understood, a study of the potential for microbes developing resistance to CO or CORMs, and a better understanding of the biological chemistry of non-heme CO targets.

Acknowledgments—We are grateful to our colleagues and especially Brian E. Mann and Thomas W. Smith for valuable discussions and reviewing this manuscript.

References


MINIREVIEW: CORMs as Antimicrobial Agents

8536 – 8542


SUPPLEMENTARY TABLE 1
CO and CORMs as antimicrobial agents: a comparison

<table>
<thead>
<tr>
<th></th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CO gas</strong></td>
<td>Stability</td>
<td>Ultimately toxic to almost all organisms</td>
</tr>
<tr>
<td></td>
<td>Adequate water solubility</td>
<td>Low toxicity against bacteria</td>
</tr>
<tr>
<td></td>
<td>Facile diffusion across membranes</td>
<td>Difficult handling and administration</td>
</tr>
<tr>
<td></td>
<td>Produced intracellularly by heme oxygenases</td>
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</tr>
<tr>
<td><strong>CORMs (generalized properties)</strong></td>
<td>Toxicity against bacteria (e.g. CORM-2 and CORM-3)</td>
<td>Complex chemistry</td>
</tr>
<tr>
<td></td>
<td>Easy handling and administration</td>
<td>Lack of knowledge on biological fates</td>
</tr>
<tr>
<td></td>
<td>CO release can be controlled</td>
<td>Only some CORMs have been tested microbiologically</td>
</tr>
<tr>
<td></td>
<td>(e.g. PhotoCORMs and enzyme-activated CORMs)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>New CORMs with desirable therapeutic effects are appearing</td>
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</table>
SUPPLEMENTARY FIGURE 1
Antibiotics and CORMs have distinct cellular targets
CO-releasing Metal Carbonyl Compounds as Antimicrobial Agents in the Post-antibiotic Era
Lauren K. Wareham, Robert K. Poole and Mariana Tinajero-Trejo

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